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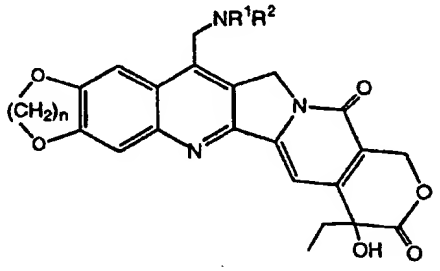

(I)

(57) Abstract

The present invention relates to water soluble, camptothecin derivatives of formula (I), wherein: n represents the integer 1 or 2; and i) R^1 represents: hydrogen, lower alkyl, (C_{3-7})cycloalkyl, (C_{3-7})cycloalkyl lower alkyl, lower alkenyl, hydroxy lower alkyl, amino lower alkyl, lower alkylamino lower alkyl, amino lower alkyl, lower alkoxy lower alkyl or $(CH_2)_tAr$ wherein: t is 0 to 5 and Ar represents phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl; or phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl, with one or more substituents independently selected from hydroxy, methoxy, halogen, and amino; and R^2 represents: diphenylmethyl or $(CH_2)_tAr$; or ii) R_1 and R_2 taken together with the linking nitrogen represent: N-tetrahydroquinolyl or N-tetrahydroisoquinolyl; and the pharmaceutically acceptable salts and solvates thereof, their use in the treatment of tumors and methods of their preparation.

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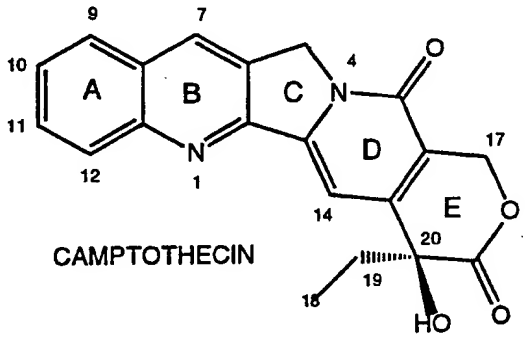
WATER SOLUBLE DERIVATIVES OF CAMPTOTHECIN AND THEIR USE AS ANTITUMOR AGENTS

The present invention relates to water soluble, camptothecin derivatives substituted in the 7 position, their use in the treatment of tumors and methods of their preparation.

BACKGROUND OF THE INVENTION

Camptothecin, a natural, cytotoxic alkaloid, is a topoisomerase I inhibitor and potent antitumor agent. It was first isolated from the leaves and bark of the Chinese plant, *Camptotheca accuminata*, by Wall, et. al. (*J. Am. Chem. Soc.*, **88** 3888 (1966)).

As depicted, camptothecin is a fused ring system, composed of a quinoline (A and B), fused to a pyrrolidine ring (C), fused to an alpha-pyridone ring (D) which in turn is fused to a lactone ring (E).



CAMPTOTHECIN

It has an asymmetric carbon at the 20 position making two enantiomeric forms possible. However, the natural occurring compound is found in the "S" configuration as shown above.

Cytotoxic agents are often employed to control or eradicate tumors i.e., they are chemotherapeutic agents. Camptothecin's cytotoxic activity is thought to be directly related to camptothecin's potency as a topoisomerase inhibitor. [For detailed explanations of the topoisomerase function see A. Lehninger, *Principles of Biochemistry*, 813, Worth Publishers, New York (1982); L. F. Liu, "DNA Topoisomerases," CRC *Critical Review in Biochemistry*, 1-24, **15** (1983) and H Vosberg, "DNA Topoisomerases: Enzymes that Control DNA Conformation," *Current Topics in Microbiology and Immunology*, 19, Springer-Verlag, Berlin

(1985).] In particular, camptothecin has been shown to be effective in the treatment of l ukemia (L-1210) and c rtain solid tumors in laboratory animals, e.g., see *Chem. Rev.* **23**, 385 (1973) and *Cancer Treat. Rep.*, **60**, 1007 (1967).

Unfortunately, in the clinic camptothecin's promise as an effective antitumor agent has not been completely fulfilled. Camptothecin is essentially insoluble in physiologically compatible, aqueous media, and must be modified to make it sufficiently soluble for parenteral administration, a preferred mode for antitumor treatment. It can be made soluble by forming its sodium salt, that is, by opening the lactone with sodium hydroxide (see F.M. Muggia, et al., *Cancer Chemotherapy Reports*, pt. 1, **56**, No.4, 515 (1972)). However, M. C. Wani, et al., *J. Med. Chem.*, **23**, 554 (1980), reported that the alpha-hydroxy lactone moiety of ring E is an absolute requirement for antitumor activity.

In the art there are examples of modifications and derivatives of camptothecin prepared to improve its solubility in water. Although many of these derivatives were active *in vitro* and in early animal studies using leukemia (L-1210) models, they were disappointing in chronic, animal models involving implanted solid tumors.

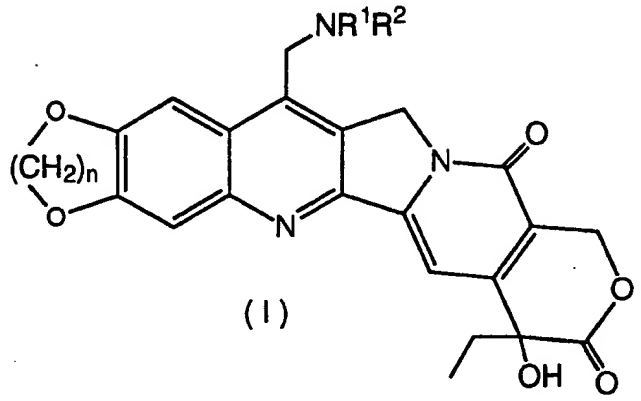
Miyasaka, et al., U.S. Patent No. 4,399,282, discloses a group of camptothecin derivatives substituted at the 7 position with, inter alia, hydroxymethyl and alkoxymethyl. Further, Miyasaka, et. al. in U.S. patent No. 4,399,276 discloses camptothecin-7-aldehyde and certain related aldehyde derivatives such as acetals, oximes and hyrazones. More recently, Vishnuvajjala, et al., in U.S. Patent No. 4,943,579, claimed a series of water-soluble camptothecin derivatives with substituents on the A ring as does Boehm, et al., European Patent Application 0 321 122 A2. Other examples of derivatives of camptothecin include Miyasaka, et al., U.S. Patent No. 4,473,692 and No. 4,545,880; and W. Kingsbury, et al., *J Med. Chem.*, **34**, 98 (1991). None of these references reported compounds with antitumor activity greater than that of camptothecin itself.

Wani and co-workers reported that 10, 11-methylenedioxycamptothecin is more potent than unsubstituted camptothecin (see M. C. Wani, et al., *J. Med. Chem*, **29**, 2358 (1986) and **30**, 2317 (1987)). However, its water solubility is as poor as camptothecin which seriously limits its clinical utility.

We have now found water-soluble analogs of camptothecin with good topoisomerase I inhibitory activity in vitro, and impressive, antitumor activity *in vivo*.

SUMMARY OF THE INVENTION

One aspect of the present invention is the water-soluble camptothecin analogs of formula (I),



(I)

wherein:

n represents the integer 1 or 2; and

i) R^1 represents:

hydrogen, lower alkyl, (C_{3-7})cycloalkyl, (C_{3-7})cycloalkyl lower alkyl, lower alkenyl, hydroxy lower alkyl, amino lower alkyl, lower alkylamino lower alkyl, amino lower alkyl, lower alkoxy lower alkyl or $(CH_2)_tAr$

wherein:

t is 0 to 5 and

Ar represents phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl; or phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl, with one or more substituents independently selected from hydroxy, methoxy, halogen, and amino; and

R^2 represents:

diphenylmethyl or$(CH_2)_tAr$; or

ii) R^1 and R^2 taken together with the linking nitrogen represent;

N-tetrahydroquinolyl or N-tetrahydroisoquinolyl;

and the pharmaceutically acceptable salts and solvates thereof.

Pharmaceutically acceptable salts include, but are not limited to salts with inorganic acids such hydrochloride, sulfate, phosphate, diphosphate, hydrobromide

and nitrat or salts with an organic acid such as acetate, malate, maleate, fumarate, tartrate, succinate, citrate, lactate, methanesulfonate, p-toluenesulfonate, palmoate, salicylate and stearate. Other acids such as oxalic, while not in themselves pharmaceutically acceptable, may be useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable salts.

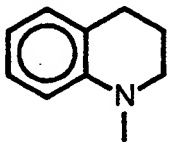
Another aspect of the invention is a method of inhibiting topoisomerase Type I in mammalian cells comprising administering to a patient a topoisomerase inhibiting amount of a compound of formula (I), and a method of treating a tumor in a mammal comprising administering to a mammal bearing a tumor, an effective antitumor amount of a compound of formula (I). A further aspect comprises pharmaceutical formulations containing a compound of formula (I) as an active ingredient. Methods of preparation of the compounds of formula (I) and the associated novel chemical intermediates used in the synthesis, as taught herein, are also within the scope of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

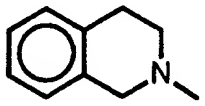
Compounds

As used herein the term "lower" in reference to alkyl and alkoxy means 1-6 carbons, especially 1-3 carbons, and in reference to alkenyl means 3-6 carbons (provided that the double bond is not attached to the carbon which is attached to the nitrogen). As use herein, the term "aryl" means aromatic ring substituents, e.g., phenyl, napthyl, furyl, pyridyl, N-methylpyrrolyl or imidazolyl. The group "$(CH_2)t$" also includes branched alkylene chains where branching is possible.

As use herein the terms "N-tetrahydroquinolyl" and "N-tetrahydroisoquinolyl" are defined as follows:



N-tetrahydroquinolyl



N-tetrahydroisoquinolyl

Th lactone ring, *i.e.*, ring E, of the camptothecin moiety may be opened by alkali metal or alkaline-earth metal bases, for example sodium hydroxide or calcium hydroxide, to form alkali metal or alkaline-earth metal salts of the

corresponding open E ring form of the compounds of formula (I). Because of its better solubility in water, the open E ring form may advantageously be purified by conventional recrystallization techniques. Accordingly, said open E ring form may then be used as an intermediate to form the compounds of formula (I), for example by treatment with acid, e.g., hydrochloric acid, and thereby produce a purified form of the compounds of formula (I).

As noted above, the camptothecin moiety has an asymmetric carbon atom at the 20 position making two enantiomeric forms, i.e., "R" and "S" configurations, possible. This invention includes both enantiomeric forms and any combinations of these forms. For simplicity, where no specific configuration at the 20 position is depicted in the structural formulas, it is to be understood that both enantiomeric forms and mixtures thereof are represented. Unless noted otherwise, the nomenclature convention, "(R,S)", denotes a racemic (approximately equal portion) mixture of the R and S enantiomers while "(R)" and "(S)" denote essential optically pure R and S enantiomers respectively. Also included in the invention are other forms of the compound of formula (I), such as solvates, hydrates, polymorphs and the like.

One sub group of compounds of the present invention are the compounds of formula (I) wherein:

n represents the integer 1 or 2; and

i) R^1 represents:

hydrogen, lower alkyl, (C_{3-7})cycloalkyl, (C_{3-7})cycloalkyl lower alkyl, lower alkenyl, hydroxy lower alkyl, amino lower alkyl, lower alkoxy lower alkyl or $(CH_2)_tAr$

wherein:

t is 0 to 5 and

Ar represents phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl; or phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl, with one or more substituents selected from hydroxy, methoxy, halogen, and amino; or

R^2 represents:

diphenylmethyl or$(CH_2)_tAr$; and

ii) R^1 and R^2 taken together with the linking nitrogen represent;

N-tetrahydroquinolyl or N-tetrahydroisoquinolyl;

and the pharmaceutically acceptable salts thereof.

Another sub group of compounds of the present invention are the compounds of formula (I) wherein:

n represents the integer 1 or 2; and

i) R^1 represents:

hydrogen, (C_{1-3}) alkyl or amino (C_{1-3}) alkyl; and

R^2 represents:

diphenylmethyl or$(CH_2)_tAr$;

wherein:

t is 1 to 3 and

Ar represents phenyl, 2-furyl, 2-pyridyl, 4-pyridyl 2-N-methylpyrrolyl, 4-imidazolyl; or phenyl, 2-furyl, 2-pyridyl, 4-pyridyl, 2-N-methylpyrrolyl, 4-imidazolyl, with one to two substituents selected from hydroxy, methoxy, halogen, and amino; or

ii) R^1 and R^2 taken together with the linking nitrogen represent N-tetrahydroisoquinolyl;

and the pharmaceutically acceptable salts thereof.

In one particular group of compounds of formula (I) Ar represents phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl or phenyl substituted with one or two substituents independently selected from hydroxy, methoxy, halogen and amino. Typical Ar groups include phenyl and substituted phenyl, 2-furyl, 2-pyridyl, 4-pyridyl, 2-N-methylpyrrolyl and 4-imidazolyl. In one particular subgroup of compounds of formula (I), Ar is substituted phenyl or 4-pyridyl.

In another subgroup of compounds R^1 represents hydrogen, lower alkyl, lower alkylaminolower alkyl or $-(CH_2)_tAr$, especially hydrogen or lower alkyl.

In another subgroup of compounds of formula (I), t is 1, 2, or 3, especially 1 and n is 1 or 2, especially 2.

Specific compounds of formula (I) are:

Example Number	Compound Name
1.	7-(N-Ethyl-N-4-pyridylmethyl)- aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
2.	7-(4-Aminobenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
3.	7-(3-Methoxy-4-hydroxybenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
4.	7-Benzylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
5.	7-(N-Tetrahydroisoquinolino)methylene-10, 11-ethylenedioxy-20(S)-camptothecin,
6.	7-Dibenzylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7.	7-(N-Methyl)benzylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
8.	7-Furylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
9.	7-(3-Phenylpropyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
10.	7-(3,4-Dimethoxybenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
11.	7-(N-Ethyl, (2-chloro-6-fluorobenzyl))-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
12.	7-(3,4-Difluorobenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
13.	7-Diphenylmethylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
14.	7-((R) 1-Phenylethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
15.	7-((S) 1-Phenylethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
16.	7-(2(N-Methyl-2-pyrrol)-ethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
17.	7-(N-benzyl-N-(2-Dimethylaminoethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
18.	7-(2-(4-Imidazolyl)ethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
19.	7-(2-Pyridylmethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
20.	7-Benzylaminomethylene-10, 11-methylenedioxy-20(S)-camptothecin, and

21. 7-(3,4-Dimethoxybenzyl)-aminomethylene-10, 11-methylenedioxy-20(S)-camptothecin, and pharmaceutically acceptable salts and solvates thereof.

Particular specific compounds of formula (I) are:

7-(N-Ethyl-N-4-pyridlymethyl)- aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,

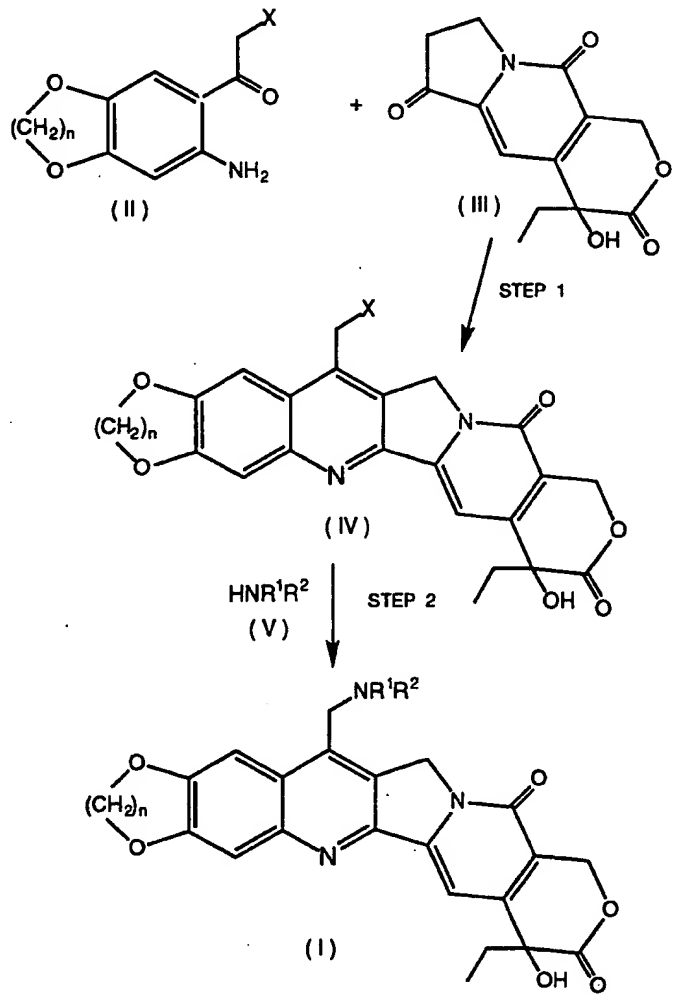
7-(4-Aminobenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,

7-(3-Methoxy-4-hydroxybenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,

and pharmaceutically acceptable salts and solvates thereof.

Preparation of Compounds

According to one general process (A), compounds of formula (I) may be prepared by the procedure shown in Step 2 of Scheme I:



SCHEME I

In Step 1 of Scheme I, a compound of formula (II), wherein X is a leaving group (as defined in J. March, *Advanced Organic Chemistry*, 3rd. Ed., page 179, John Wiley & Sons, New York (1985)), for example, a halogen, e.g., chloro, may be reacted with a compound of formula (III) according to the method taught in US Patent 4,894,456 (hereinafter, '456), issued January 16, 1990 to Wall et al., incorporated herein by reference, to yield a compound of formula (IV).

The reaction of Step 1 is preferably carried out in the presence of an acid or base catalyst. The acid catalyst is preferably a strong mineral acid, for example hydrochloric, nitric, sulfuric and phosphoric or a strong organic acid such as C_{1-8} alkanoic acids and C_{1-12} arylsulfonic acids, especially p-toluenesulfonic acid. The base catalyst is preferably an inorganic base, for example sodium and potassium carbonate and sodium and potassium bicarbonate or an organic base such as a

sterically hinder d base, for xample, triethylamine and diisopropylamine.

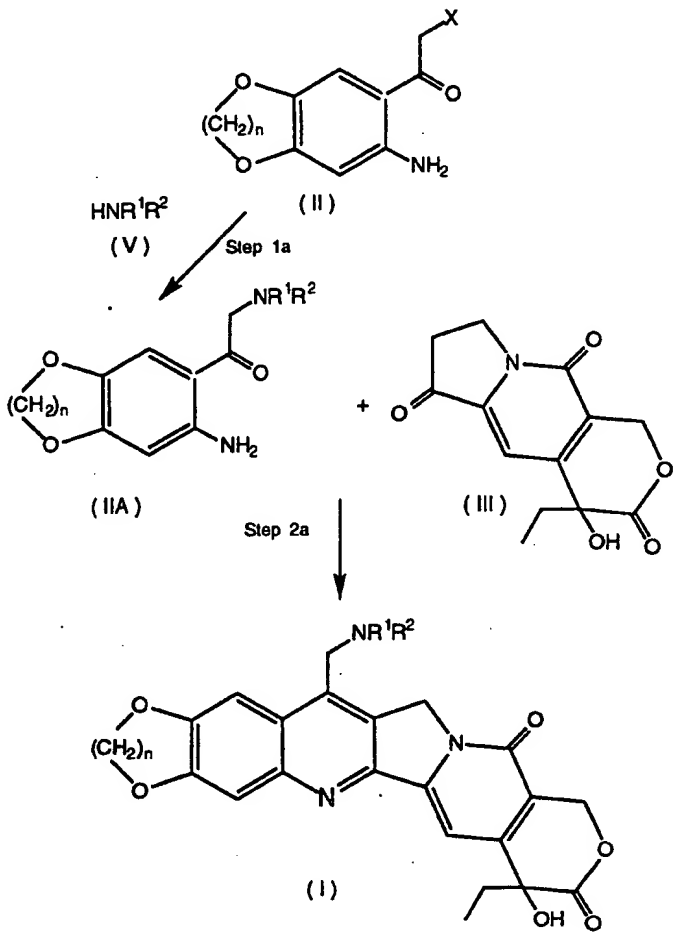
This reaction may be carried out neat or in the presence of a polar or non-polar solvent. Preferred polar solvents are C_{1-6} alcohols, C_{1-6} ethers, and dimethylformamide. Preferred non-polar solvents are branched or straight chained alkyl hydrocarbons having 4-10 carbon atoms and aromatic hydrocarbons having 6-20 carbon atoms especially toluene. The reaction is generally conducted with heating at reflux.

In Step 2, (General Process A) the compounds of formula (IV) may be converted to the compounds of formula (I) by displacement of the leaving group, X, with a compound of formula (V), wherein R^1 and R^2 are as defined for formula (I). This displacement reaction may conveniently be carried out in a solvent system, for example water, a (C_{1-4}) alkanol, a (C_{2-4}) alkylene diol, 1-hydroxy-2-methoxyethane, dimethylacetamide (DMAC), N-methylpyrolidinone, dimethyl formamide (DMF), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), toluene, dioxane or a combination of these solvents in the presence of excess amine, i.e., excess compound of formula (V), with or without a base, e.g., potassium carbonate, and/or with NaI as a catalyst.

This method is particularly useful for preparing compounds of formula (I) wherein R^1 is other than hydrogen.

Compounds of formula (V) are commercially available, taught in the chemical literature, or may be readily prepared by one skilled in the art of organic chemistry using methods and materials known in the art of organic chemistry.

According to another general process (B) , compounds of formula (I) may be prepared by the procedure shown in Step 2a of Scheme IA:

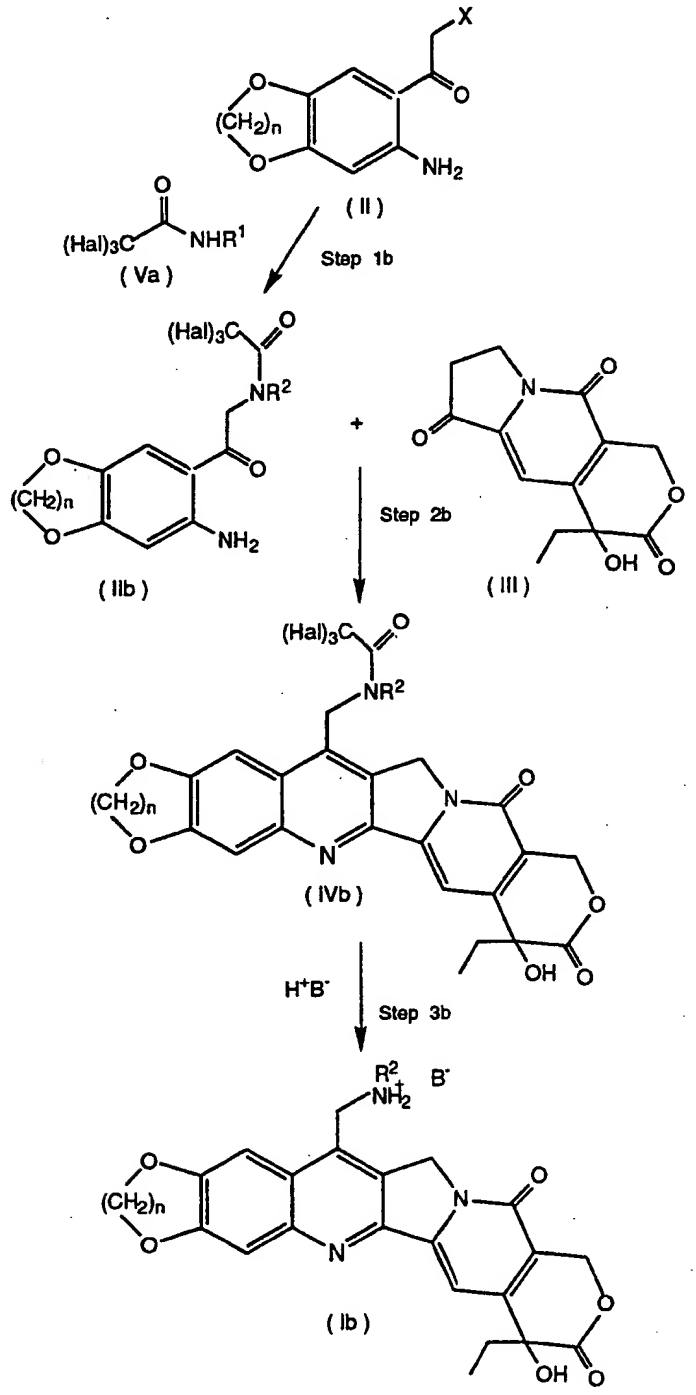


SCHEME 1A

In Step 1a, a compounds of formula (II) may be converted to a compounds of formula (IIA) by displacement of the leaving group, X (as defined for Scheme I), with a compound of formula (V), wherein R^1 and R^2 are as defined for formula (I). This displacement reaction may conveniently be carried out in a solvent system, for example, water, a (C_{1-4}) alkanol, a (C_{2-4}) alkylene diol, 1-hydroxy-2-methoxyethane, dimethylacetamide (DMAC), N-methylpyrolidinone, dimethyl formamide (DMF), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), toluene, dioxane or a combination of these solvents (to the extent of miscibility) in the presence of excess amine, i.e., excess compound of formula (V), with or without a base, e.g., potassium carbonate, and/or with NaI as a catalyst.

In Step 2a (General Process B), compound of formula (IIA) is reacted with a compound of formula (III) in a similar manner to that taught above in Scheme 1, Step 1, to yield a compound of formula (I).

Further alternate general method (C), particularly useful for preparing compounds of Formula (I) wh r in R^1 is hydrogen, is shown in Step 3b of Scheme 1B.



SCHEME 1B

In Step 1b, a compound of formula (Va) (wherein "Hal" is halogen, i.e., fluoro, chloro, bromo or iodo) e.g., trifluoroacetamide, is reacted with a compound of formula (II) in a polar, aprotic solvent, e.g., acetonitrile, in the presence of a base soluble in the polar, aprotic solvent, e.g., cesium carbonate if the solvent is acetonitrile, to yield a compound of formula (IIb).

In Step 2b, a compound of formula (IIb) is reacted with a compound of formula (III) in a similar manner to that taught in Scheme 1, Step 1, to yield a compound of formula (IVb).

In Step 3b (General Process C), a compound of formula (IVb) is treated with an acid, H^+B^-, such as a mineral acid, e.g., hydrochloric acid, to yield a compound of formula (Ib), i.e. salt of a compound of formula (I). The compound of formula (Ib) may be treated with a base, such as an alkali metal hydroxide or carbonate, e.g., sodium hydroxide or potassium carbonate, by standard method of the art to yield the corresponding free base. For example a compound of formula (Ib) may be stirred with an aqueous solution of potassium carbonate for about one to about four hours in the temperature range of from about 5^o to about 100^oC. The free base can then be converted by conventional means to a pharmaceutically acceptable salt if required.

The compounds of formula (II) and (III) may be prepared according to the procedure described in EP0 540 099 A1.

The novel, intermediate compounds of formulas (IIa), (IIb) and (IVb) are within the scope of this invention.

A compound of formula (I) according to the invention may be converted into another compound of the invention using conventional procedures.

Thus, for example, a compound of formula (I) wherein R^1 represents a hydrogen atom, may be alkylated using conventional techniques. The reaction may be effected using a suitable aklylating agent such as an alkyl halide, an alkyl tosylate or a dialkylsulphate. The alkylation reaction may conveniently be carried out in an organic solvent such as an amide, e.g. dimethylformamide, or an ether, e.g. tetrahydrofuran, preferably in the presence of a base. Suitable bases include, for example, alkali metal hydrides, such as sodium hydride, alkali metal carbonates, such as sodium carbonate, or potassium methoxide, ethoxide or t-butoxide. The alkylation reaction is conveniently carried out at a temperature of

from about 25 to about 100°C.

Alternately, a compound of formula (I) wherein R^1 represents a hydrogen atom may be converted to another compound of formula (I) by reductive alkylation. Reductive alkylation with an appropriate aldehyde or ketone may be effected using an alkaline earth metal borohydride or cyanoborohydride. The reaction medium, conveniently in an alcohol, e.g. methanol or ethanol or an ether, e.g. dioxan or tetrahydrofuran, optionally in the presence of water. The reaction may conveniently be carried out at a temperature in the range of 0 to 100°C, preferably about 5 to about 50°C.

Alternatively, a compound of formula (I) wherein R^1 represents a lower alkenyl group may be converted to another compound of formula (I) wherein R^1 represents a lower alkyl group. Reduction may conveniently be effected in the presence of hydrogen and a metal catalyst, for example, Raney nickel or a nobel metal catalyst such as palladium, platinum, platinum oxide or rhodium, which may be supported, for example, on charcoal. The reaction may be effected in a solvent such as an alcohol, for example ethanol and conveniently at a temperature of from about -10 to about +50°C, preferably about 20 to about 30°C.

A compound of formula (I) according to the invention, or a salt thereof may br prepared by subjecting a protected derivative of formula (I) or a salt thereof to reaction to remove the protecting group or groups.

Thus, at an earlier stage in the preparation of a compound of formula (I) or a salt thereof it may have been necessary and/or desirable to protect one or more sensitive groups in the molecule to prevent undesirable side reactions.

The protecting groups used in the preparation of compounds of formula (I) may be used in conventional manner. See for example, "Protective Groups in Organic Chemistry" Ed. J.F.W. McOmie (Plenum Press 1973) or "Protective Groups in Organic Synthesis" by Theodora W. Greene (John Wiley and Sons 1981).

Conventional amino protecting groups may include, for example, aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl groups; and acyl groups such as N-benzyloxycarbonyl or t-butoxycarbonyl. Thus, compounds of general formula (I) wherein R^1 represents hydrogen may be prepared by deprotection of a corresponding protected compound.

Hydroxy groups may be protected, for example, by aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl groups, acyl groups, such as acetyl, silicon protecting groups, such as trimethylsilyl or t-butyl dimethylsilyl groups or as tetrahydropyran derivatives.

Removal of any protecting groups present may be achieved by conventional procedures. Thus, an aralkyl groups such as benzyl, may be cleaved by hydrogenolysis in the presence of a catalyst (e.g. palladium on charcoal); an acyl group such as N-benzyloxycarbonyl may be removed by hydrolysis with, for example, hydrogen bromide in acetic acid or by reduction, for example by catalytic hydrogenation; silicon protecting groups may be removed, for example, by treatment with fluoride ion or by hydrolysis under acidic conditions; tetrahydropyran groups may be cleaved by hydrolysis under acidic conditions.

As will be appreciated, in any of the processes described above, it may be necessary or desired to protect any sensitive groups in the molecule as just described. Thus, a reaction step involving deprotection of a protected derivative of general formula (I) or a salt thereof may be carried out subsequent to any of the above described processes.

Thus, according to a further aspect of the invention, the following reactions may, if necessary and/or desired by carried out in any appropriate sequence subsequent to any of the processes

(i) removal of any protecting groups; and

(ii) conversion of a compound of formula (I) or a salt thereof into a pharmaceutically acceptable salt thereof.

Where it is desired to isolate a compound of the invention as a salt, for example, as an acid addition salt, this may be achieved by treating the free base of general formula (I) with any appropriate acid, preferably with an equivalent amount, or with creatinine sulphate in a suitable solvent (e.g. aqueous ethanol).

As well as being employed as the last main step in the preparative sequence, the general methods indicated above for the preparation of the compounds of the invention may also be used of the introduction of the desired groups at an intermediate stage in the preparation of the required compound. It should therefore be appreciated that in such multi-stage processes, the sequence of reactions should be chosen in order that the reacting conditions do not affect groups present in the

molecule which are desired in the final product.

The biological activity of the compounds of formula (I) appears to reside in the S enantiomer, and the R enantiomer has little or no activity. Thus, the S enantiomer of a compound of formula (I) is generally preferred over a mixture of R and S such as the racemic mixture. However, if the R enantiomer were desired, e.g., for control studies or synthesis of other compounds, it could be conveniently prepared by the procedure above using the R enantiomer of the compound of formula (III) prepared according to the teachings of '512.

A compound of formula (I) prepared by reaction Scheme I or Scheme IA may be purified by conventional methods of the art, e.g., chromatography, distillation or crystallization.

Cleavable Complex *in vitro* Assay

The data in Table A, below, shows the relative topoisomerase Type I inhibitory activity of the compounds of Formula (I). This assay performed according to the method described in Hsiang, Y. et al., *J. Biol. Chem.*, 260:14873-14878 (1985), correlates well with in vivo anti-tumor activity of topoisomerase inhibitors in animal models of cancer, e.g., camptothecin and its analogs. See Hsiang et al., *Cancer Research*, 49:4385-4389 (1989) and Jaxel et al., *Cancer Research*, 49:1465-1469 (1989).

Those compounds which exhibit observable activity at concentrations greater than 2000 nM ("+" in Table A) are considered weakly to moderately active, while those with activity at concentrations less than 500 nM ("++++" in Table A) are very active. The term "IC_{50}" means the concentration of a compound of formula (I) at which 50% of the DNA substrate has been captured by topoisomerase I.

TABLE A

Topoisomerase Inhibitory Activity of
Compounds of Formula (I) in the Cleavable Complex Assay

Example Number	Isomeric form	Relative IC_{50}*
4	(S)	++++
8	(S)	++++
1	(S)	++++
14	(S)	++++
15	(S)	++++

	16	(S)	++++
	21	(S)	++++
	2	(S)	++++
	19	(S)	++++
5	3	(S)	++++
	20	(S)	++++
	18	(S)	++++
	6	(S)	+++
	7	(S)	+++
10	10	(S)	+++
	12	(S)	+++
	17	(S)	+++
	5	(S)	++
	11	(S)	++
15	13	(S)	++
	9	(S)	++

*IC_{50} Range	Symbol	nM
	++++	<~500
20	+++	<~1000>~500
	++	<~2000>~1000
	+	>~2000

Utility

25

In view of such activity, the compounds of formula (I) are active against a wide spectrum of mammalian (including human) tumors and cancerous growths such as cancers of the oral cavity and pharynx (lip, tongue, mouth, pharynx), esophagus, stomach, small intestine, large intestine, rectum, liver and biliary passages, pan-
30 creas, larynx, lung, bone, connective tissue, skin, colon, breast, cervix uteri, corpus endometrium, ovary, prostate, testis, bladder, kidney and other urinary tissues, eye, brain and central nervous system, thyroid and other endocrine gland, leukemias (lymphocytic, granulocytic, monocytic), Hodgkin's disease, non-Hodgkin's lymphomas, multiple myeloma, etc. Hereinf the terms "tumor", "cancer" and "cancerous
35 growths" are used synonymously.

The amount of compound of formula (I) required to be effective as an antitumor agent will, of course, vary with the individual mammal being treated and is ultimately at the discretion of the medical or veterinary practitioner. The factors to be

considered include the condition being treated, the route of administration, the nature of the formulation, the mammal's body weight, surface area, age and general condition, and the particular compound to be administered. However, a suitable effective antitumor dose is in the range of about 0.1 to about 200 mg/kg body weight per day, preferably in the range of about 1 to about 100 mg/kg per day. The total daily dose may be given as a single dose, multiple doses, e.g., two to six times per day, or by intravenous infusion for a selected duration. Dosages above or below the range cited above are within the scope of the present invention and may be administered to the individual patient if desired and necessary.

For example, for a 75 kg mammal, a dose range would be about 75 to about 7500 mg per day, and a typical dose would be about 800 mg per day. If discrete multiple doses are indicated, treatment might typically be 200 mg of a compound of formula (I) given 4 times per day.

Formulations

Formulations of the present invention, for medical use, comprise an active compound, i.e., a compound of formula (I), together with an acceptable carrier therefof and optionally other therapeutically active ingredients. The carrier must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient therefor.

The present invention, therefore, further provides a pharmaceutical formulation comprising a compound of formula (I) together with a pharmaceutically acceptable carrier thereof.

The formulations include those suitable for oral, rectal or parenteral (including subcutaneous, intramuscular and intravenous) administration. Preferred are those suitable for oral or parenteral administration.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active compound into association with a carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier or a finely divided solid carrier and then, if necessary, shaping the product into desired unit dosage form.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the active compound; as a powder or granules; or a suspension or solution in an aqueous liquid or non-aqueous liquid, e.g., a syrup, an elixir, an emulsion or a draught.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form, e.g., a powder or granules, optionally mixed with accessory ingredients, e.g., binders, lubricants, inert diluents, surface active or dispersing agents. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered active compound with any suitable carrier.

A syrup or suspension may be made by adding the active compound to a concentrated, aqueous solution of a sugar, e.g., sucrose, to which may also be added any accessory ingredients. Such accessory ingredient(s) may include flavoring, an agent to retard crystallization of the sugar or an agent to increase the solubility of any other ingredient, e.g., as a polyhydric alcohol, for example, glycerol or sorbitol.

Formulations for rectal or vaginal administration may be presented as a suppository with a conventional carrier, e.g., cocoa butter or Witepsol S55 (trademark of Dynamite Nobel Chemical, Germany, for a suppository base).

For transdermal administration, the compounds according to the invention may be formulated as creams, gels, ointments or lotions or as a transdermal patch. Such compositions may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening, gelling, emulsifying, stabilizing, dispersing, suspending and/or coloring agents.

Formulations suitable for parenteral administration conveniently comprise a sterile aqueous preparation of the active compound which is preferably isotonic with the blood of the recipient. Such formulations suitably comprise a solution or suspension of a pharmaceutically and pharmacologically acceptable acid addition salt of a compound of the formula (I) that is isotonic with the blood of the r cipient. Thus, such formulations may conveniently contain distilled water, 5% dextrose in distilled water or saline and a pharmaceutically and pharmacologically acceptable acid addition salt of a compound of the formula (I) that has an appropriate solubility

in these solvents, for xample the hydrochloride. Useful formulations also comprise concentrated solutions or solids containing the compound of formula (I) which upon dilution with an appropriate solvent give a solution suitable for parental administration above.

In addition to the aforementioned ingredients, the formulations of this invention may further include one or more optional accessory ingredient(s) utilized in the art of pharmaceutical formulations, e.g., diluents, buffers, flavoring agents, binders, surface active agents, thickeners, lubricants, suspending agents, preservatives (including antioxidants) and the like.

EXAMPLES

The following examples illustrate aspects of this invention but should not be construed as limitations. The symbols and conventions used in these examples are consistent with those used in the contemporary chemical literature, for example, the Journal of the American Chemical Society. As used here in the term "room temperature" means about 25°C.

GENERAL PROCEDURE

A flask is charged with (S)-7-chloromethyl-10,11-ethylenedioxycamptothecin (25-150 mg, 0.06-0.33 mmol), catalytic sodium iodide (1-15 mg, 99.99% Aldrich), and anhydrous 1,4-dioxane (2-50 mL). The amine is added (2-5 equiv. neat), and the stirred slurry was heated in an oil bath at 75-95 °C. The reaction was monitored for disappearance of starting material by thin layer chromatography. The mixture is worked up by removing the solvent with a rotary evaporator, and triturating the residue with ether. The solid that is collected by suction filtration is purified by silica gel chromatography or reverse phase HPLC (Rainin Dynamax 60A column, eluting with 2% trifluoroacetic acid in water (70-80%) and 4:1 acetonitrile THF (20-30%), monitoring at 254 nm) to afford the product amine as either the free base or the trifluoroacetic acid salt respectively.

Example 1

7-(N-Ethyl-N-4-pyridylmethyl)- aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin (Compound 1)

(A) 6'-amino-3',4'-ethylenedioxy-2-chloroacetophenone

A 1-L, three-necked, round-bottomed flask was fitted with a magnetic stirring bar, thermometer, reflux condenser with calcium chloride filled drying tube, and a nitrogen inlet. The reaction vessel was charged with dry methylene chloride (100 ml) and 1,4-benzodioxane-6-amine (15.12 g, 100 mmol). The reaction vessel was cooled to 0°C followed by slow addition of 400 ml of a 1M solution of boron trichloride in methylene chloride while maintaining an internal temperature at or below 10°C. Aluminum chloride (13.34 g, 100 mmol) was added quickly in three portions followed by addition of chloroacetonitrile (7 ml, 110 mmol). The reaction was stirred for 30 min at 0°C then heated to 40°C for 16 hours. The reaction was removed from heat, allowed to cool to room temperature, then quenched into a mixture of 1 kg of ice/ 500 ml of 1N HCl. The mixture was stirred until no solids were observed. The methylene chloride layer was removed and the aqueous layer was extracted twice with methylene chloride. The organic layers were combined, washed with brine, dried over magnesium sulfate, filtered, treated with decolorizing carbon, filtered through a pad of celite, and concentrated to a solid residue. The solid was recrystallized from ethyl acetate/hexanes to give 8.3 g (36.5%) of 6'-amino-3',4'-ethylenedioxy-2-chloroacetophenone.

MS (CI): m/z 228 (M+H+).

1H NMR ($CDCl_3$):δ 7.14 (s, 1H), 6.15 (s, 1H), 4.57 (s, 2H), 4.3 (m, 2H), 4.2 (m, 2H), 1.6 (s, broad, 2H).

Anal. ($C_{10}H_{10}ClNO_3$)

	calc.	found
C	52.76	52.66
H	4.43	4.53

7-Chloromethyl-10,11-ethylenedioxy-20(S)-camptothecin. Into a 250 ml round-bottomed flask equipped with a stirring bar, a reflux condeser and a Dean Stark

trap were added 1.82 g (8.0 mmol) of 2'-amino-4',5'-ethylenedioxy-2-chloroacetophenone, 40 ml dry toluene and 2.0 g (7.6 mmol) of (S) tricyclic keto-lactone. The reaction was stirred under nitrogen and refluxed for 0.5 hours. The reaction was allowed to cool followed by addition of 100 mg, (0.53 mmol) of p-toluenesulfonic acid. The reaction was then heated to reflux for 36 hours. The reaction was cooled, and the solids were collected by filtration and washed with toluene followed by thorough washings of the solids by anhydrous ethanol. The remaining greenish-brown solid was dried under vacum at room temperature yielding 2.67 g (77.2%) of > than 97% pure materal.

1H NMR (DMSO-d6): δ 7.47 (s,1H), 7.32 (s, 1H), 6.98 (s, 1H), 5.15 (s, 1H), 5.06 (s, 1H), 4.97 (s, 1H), 4.19 (s, 4H), 2.23 (s, 2H), 1.6 (m, 2H), 0.68 (m, 3H).

MS M+H = 455.

HRMS: Calc. 455.1009; Found 455.1000.

Anal. ($C_{23}H_{19}N_2ClO_6$) C, H

	calc.	found
C	60.73	60.70
H	4.21	4.30

(C) 7-(N-Ethyl-N-4-pyridylmethyl)- aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin

A flask is charged with (S)-7-chloromethyl-10,11-ethylenedioxycamptothecin (0.60 g,1.3 mmol), catalytic sodium iodide (20 mg, 99.99% Aldrich), and anhydrous 1,4-dioxane (120 mL). The 4-(ethylaminomethyl)pyridine (0.54 g, 4.0 mmol) is added and the stirred slurry was heated in an oil bath at 75-95 °C. The reaction was monitored for disappearance of starting material by thin layer chromatography. The mixture is worked up by removing the solvent with a rotary evaporator, and triturating the residue with ether. The solid that is collected by suction filtration is purified by reverse phase HPLC (Rainin Dynamax 60A column, eluting with 2% trifluoroacetic acid in water (70-80%) and 4:1 acetonitrile:THF (20-30%), monitoring at 254 nm) to afford the product amine as the trifluoroacetic acid salt.

Mp 178-180°C.

FAB MS MH^+ 555.

^1H NMR (d^6-DMSO) δ 8.58(d, 2H), 7.83(s, 1H), 7.58(d, 2H), 7.52(s, 1H), 7.24(s, 1H), 5.42(s, 2H), 5.31(s, 2H), 4.46(s, 4H), 4.15(br s, 2H), 3.79(br s, 2H), 2.65(m, 2H), 1.86(m, 2H), 1.16(t, 3H), 0.87(t, 3H).

Example 2

7-(4-aminobenzyl)aminomethyl-10,11-ethylenedioxy-(20S)-camptothecin (Compound 2)

A flask is charged with 7-chloromethyl-10,11-ethylenedioxy-(20S)-camptothecin (252 mg, 0.555 mmol), NaI (42 mg, 0.307 mmol) and anhydrous 1,4-dioxane (2-50 mL). 4-Aminobenzylamine (192 mL, 207 mg, 1.7 mmol), is added and the stirred slurry is heated in an oil bath at 75-95 °C. The reaction is monitored for disappearance of starting material by thin layer chromatography. The mixture is worked up by removing the solvent with a rotary evaporator, and triturating the residue with ether. The crude product is collected by suction filtration and purified by flash chromatography (eluting with 6:5:1 EtOAc/$CHCl_3$/MeOH) to afford the amine product which was further purified by recrystallizing from *t*-butylmethyl ether to afford 100.6 mg (34% yield) of pure product. The TLC salt was formed by dissolving in 2% aqueous trifluoroacetic acid and lyophylizing to afford the product TFA salt (141.4 mg) as a bright yellow solid, mp 280 °C (dec).

^1H NMR (300 MHz, DMSO-d_6): δ 0.85 (t, 3H, J=7.1), 1.78-1.95 (m, 2H), 4.27 (bs, 1H), 4.45 (s, 2H), 4.65 (bs, 1H), 5.42 (s, 2H), 6.63 (d, 1H, J=8.30), 7.21 (d, 1H, J=8.30), 7.27 (s, 1H), 7.63 (s, 1H), 7.75 (s, 1H), 9.05 (bs, 2H).

Low resolution ms (M+1): 541.3 (calcd 541).

Anal. Calcd $C_{30}H_{28}N_4O_8 \cdot 2TFA \cdot 2.5H_2O$:

	calc.	found
C	48.23	48.25
H	4.22	4.18
N	6.43	6.39.

Example 3

7-(3-Methoxy-4-hydroxybenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin (Compound 3)

A flask is charged with (S)-7-chloromethyl-10,11-ethylenedioxycamptothecin (250 mg, 0. 549 mmol), catalytic sodium iodide (20 mg, 99.99% Aldrich), and anhydrous 1,4-dioxane (25 mL). 4-Hydroxy-3-methoxybenzylamine is added (254 mg,1.66 mmol neat), and the stirred slurry was heated in an oil bath at 75-95 °C for 18h. The reaction was monitored for disappearance of starting material by thin layer chromatography. The mixture is worked up by removing the solvent with a rotary evaporator, and triturating the residue with ether. The solid that is collected by suction filtration is purified by reverse phase HPLC (Rainin Dynamax 60A column, eluting with 2% trifluoroacetic acid in water (70-80%) and 4:1 acetonitrile THF (20-30%), monitoring at 254 nm) to afford the product amine as the trifluoroacetic acid salt 214 mg (68%).

MS (FAB) (M+H) $^{+}$572.3.

^{1}H NMR (DMSO-d6): δ 7.74 (s,1H), 7.63 (s,1H), 7.28 (s,1H), 7.24 (br s,1H), 6.95 (d,1H,j=8Hz), 6.82 (d,1H,j=8Hz), 5.42 (s,2H), 4.68 (br s,1H), 4.46 (br s,2H), 4.35 (br s,1H), 3.78 (s,3H), 1.86 (m,2H), 0.85 (t,2H).

Examples 4-21

The following compounds of formula (I) are prepared by the procedure taught in Scheme I, in an analogous manner to Example 1 using the appropriate intermediate compounds of formulas (II), (III), and (V).

4. 7-Benzylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as the trifluoroacetic acid (TFA) salt)
Nominal mass spectrum: MH+526,
mp = >300°C (decomposition).

5. 7-(N-tetrahydroisoquinolino)methylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+552,
mp = 265-270°C.

6. 7-Dibenzylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+630,
mp = 180°C (decomposition).

7. 7-(N-Methyl)benzylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+540,
mp = 205°C (decomposition).

8. 7-Furylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+516,
mp = 185°C (decomposition).

9. 7-(3-Phenylpropyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+554,
mp = 280°C (decomposition).

10. 7-(3,4-Dimethoxybenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+586,
mp = 190°C.

11. 7-(N-Ethyl, (2-chloro-6-fluorobenzyl))-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+606,
mp = 172°C (decomposition).

12. 7-(3,4-Difluorobenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+562,
mp = 274-277°C.

13. 7-Diphenylmethylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+602,
mp = 250°C (decomposition).

14. 7-((R) 1-Phenylethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+540,
mp = 265-270°C.

15. 7-((S) 1-Phenylethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+552,
mp = 265-270°C.

16. 7-(2(N-Methyl-2-pyrrol)-ethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+552,
mp = 160°C (decomposition).

17. 7-(N-benzyl-N-(2-Diethylaminoethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+597,
mp = 170-173°C.

18. 7-(2-(4-Imidazolyl)ethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+530,
mp = 190°C.

19. 7-(2-Pyridylmethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin, (as TFA salt)
Nominal mass spectrum: MH+527,
mp = >300°C.

20. 7-Benzylaminomethylene-10, 11-methylenedioxy-20(S)-camptothecin, (as the hydrochloride salt
Nominal mass spectrum: MH+512,
mp = >256°C (decomposition).

21. 7-(3,4-Dimethoxybenzyl)-aminomethylene-10, 11-methylenedioxy-20(S)-camptothecin, (as the hydrochloride salt)
Nominal mass spectrum: MH+472,
mp = 225°C (decomposition).

Example 22

Pharmaceutical formulations

(A) Transdermal System

Ingredients	Amount
Active compound	600.0 mg
Silicone fluid	450.0 mg
Colloidal silicone dioxide	25.0 mg

The silicone fluid and active compound, i.e., a compound of formula (I), are mixed together and the colloidal silicone dioxide is reacted with to increase viscosity. The material is then dosed into a subsequently heat sealed polymeric laminate comprised of the following: polyester release liner, skin contact adhesive composed of silicone or acrylic polymers, a control membrane which is a polyolefin (e.g. polyethylene),polyvinyl acetate or polyurethane, and an impermeable backing membrane made of a polyester multilaminate. The system described is a 10 sq. cm patch.

(B) Oral Tablet

Ingredients	Amount
Active compound	200.0 mg
Starch	20.0 mg
Magnesium Stearate	1.0 mg

The active compound and the starch are granulated with water and dried. Magnesium stearate is added to the dried granules and the mixture is thoroughly blended. The blended mixture is compressed into a tablet.

(C) Suppository

Ingredients	Amount
Active compound	150.0 mg
Theobromine sodium salicylate	250.0 mg
Witepsol S55	1725.0 mg

The inactive ingredients are mixed and melted. The active compound is then

distributed in the molten mixture, poured into molds and allowed to cool.

(D) Injection

Ingredients	Amount
Active Compound	20.0 mg
Buffering Agents	q.s.
Propylene glycol	0.4
Water for injection	0.6 mL

The active compound and buffering agents are dissolved in the propylene glycol at about 50°C. The water for injection is then added with stirring and the resulting solution is filtered, filled into an ampule, sealed and sterilized by autoclaving.

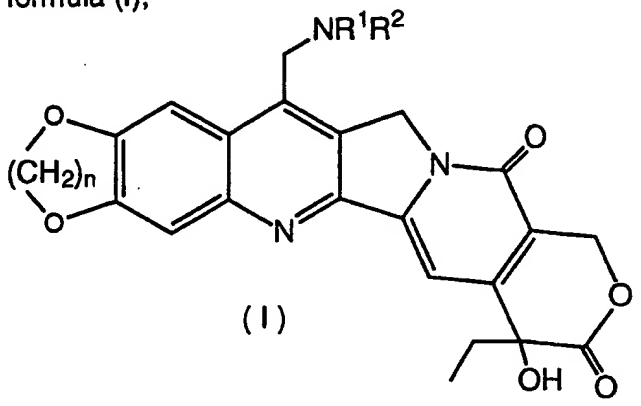
(E) Capsule

Ingredients	Amount
Active Compound	200.0 mg
Lactose	450.0 mg
Magnesium stearate	5.0 mg

The finely ground active compound is mixed with the lactose and stearate and packed into a gelatin capsule.

W claim:

1. A compound of formula (I),



(I)

wherein n represents the integer 1 or 2; and

i) R^1 represents:

hydrogen, lower alkyl, (C_{3-7})cycloalkyl, (C_{3-7})cycloalkyl lower alkyl, lower alkenyl, hydroxy lower alkyl, amino lower alkyl, lower alkylamino lower alkyl, amino lower alkyl, lower alkoxy lower alkyl or $(CH_2)_tAr$

wherein:

t is 0 to 5 and

Ar represents phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl; or phenyl, furyl, pyridyl, N-methylpyrrolyl, or imidazolyl with one or more substituents independently selected from hydroxy, methoxy, halogen, and amino; and

R^2 represents:

diphenylmethyl or$(CH_2)_tAr$; or

ii) R^1 and R^2 taken together with the linking nitrogen represent; N-tetrahydroquinolyl or N-tetrahydroisoquinolyl;

or a pharmaceutically acceptable salt or solvate thereof.

2. A compound according to Claim 1 wherein Ar represents phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl or phenyl substituted with one or two substituents independently selected from hydroxy, methoxy, halogen and amino.

3. A compound according to Claim 1 or Claim 2 wherein Ar represents phenyl or substituted phenyl, 2-furyl, 2-pyridyl, 4-pyridyl, 2-N-methylpyrrolyl or 4-imidazolyl.

4. A compound according to any one of Claims 1 to 3 wherein Ar represents substituted phenyl or 4-pyridyl.

5. A compound according to any one of Claims 1 to 4 wherein R^1 represents hydrogen, lower alkyl, lower alkylamino lower alkyl or $-(CH_2)_tAr$.

6. A compound according to any one of Claims 1 to 5 wherein R^1 represents hydrogen or lower alkyl.

7. A compound according to any one of Claims 1 to 6 wherein t is 1, 2 or 3.

8. A compound according to any one of Claims 1 to 7 wherein t is 1.

9. A compound according to any one of Claims 1 to 8 wherein n is 1 or 2.

10. A compound according to any one of Claims 1 to 9 wherein n is 1.

11. The compound which is:
7-(N-Ethyl-4-pyridylmethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin;
7-(4-Aminobenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin; or
7-(3-Methoxy-4-hydroxybenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin;
or a pharmaceutically acceptable salt or solvate thereof.

12. A compound according to Claim 1 wherein n represents the integer 1 or 2; and
i) R^1 represents hydrogen, lower alkyl, (C_{3-7})cycloalkyl, (C_{3-7})cycloalkyl lower alkyl, lower alkenyl, hydroxy lower alkyl, amino lower alkyl, lower alkoxy lower alkyl or $(CH_2)_tAr$ wherein t is 0 to 5 and Ar represents phenyl, furyl, pyridyl, N-methylpyrrolyl or imidazolyl; or phenyl, furyl, pyridyl, N-methylpyrrolyl or imidazolyl with one or more substituents selected from hydroxy, methoxy, halogen and amino; and
R^2 represents diphenylmethyl or $(CH_2)_tAr$; or
ii) R^1 and R^2 taken togeth r with the linking nitrogen represent N-tetrahydroquinolyl or N-tetrahydroisoquinolyl.

13. A compound according to Claim 1 wherein n represents the integer 1 or 2; and

i) R^1 represents hydrogen, (C_{1-3}) alkyl or amino (C_{1-3}) alkyl; and R^2 represents diphenylmethyl or $(CH_2)_tAr$ wherein t is 1 to 3 and Ar represents phenyl, 2-furyl, 2-pyridyl, 4-pyridyl, 2-N-methylpyrrolyl, 4-imidazolyl; or phenyl, 2-furyl, 2-pyridyl, 4-pyridyl, 2-N-methylpyrrolyl or 4-imidazolyl with one to two substituents selected from hydroxy, methoxy, halogen and amino; or

ii) R^1 and R^2 taken together with the linking nitrogen represent N-tetrahydroisoquinolyl.

14. A compound according to any one of Claims 1 to 13 in the R configuration.

15. A compound according to any one of Claims 1 to 13 in the S configuration.

16. A compound according to any one of Claims 1 to 14 for use in therapy.

17. A pharmaceutical composition comprising a compound according to any one of Claims 1 to 14 or pharmaceutically acceptable salt or solvate thereof, together with a pharmaceutically acceptable carrier.

18. A method of inhibiting topoisomerase I enzyme comprising contacting said enzyme with an effective topoisomerase I inhibitory amount of a compound according to any one of Claims 1 to 14.

19. A method of treating a tumor in a mammal comprising administering to said mammal an antitumor effective amount of a compound according to any one of Claims 1 to 14.

20. The use of a compound according to any one of Claims 1 to 14 in the preparation of a medicament for use in the treatment of tumors.

21. The compound of Claim 1 which is:

7-(N-Ethyl-N-4-pyridylomethyl)- aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,

7-(4-Aminobenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,

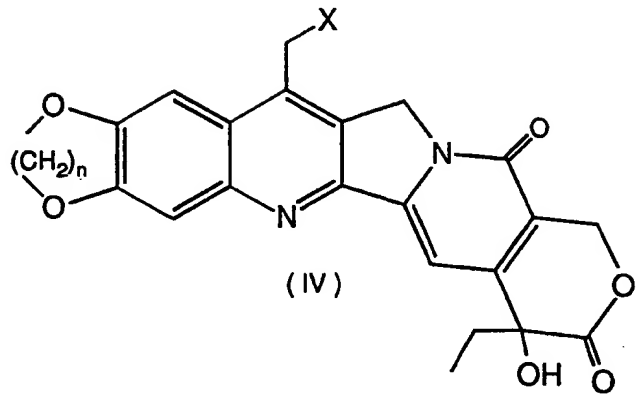
7-(3-Methoxy-4-hydroxybenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,

7-Benzylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,

7-(N-T trahydroisoquinolino)methylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-Dibenzylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-(N-Methyl)benzylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-Furylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-(3-Phenylpropyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-(3,4-Dimethoxybenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-(N-Ethyl, (2-chloro-6-fluorobenzyl))-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-(3,4-Difluorobenzyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-Diphenylmethylaminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-((R) 1-Phenylethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-((S) 1-Phenylethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-(2(N-Methyl-2-pyrrol)-ethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-(N-benzyl-N-(2-Dimethylaminoethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-(2-(4-Imidazolyl)ethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-(2-Pyridylmethyl)-aminomethylene-10, 11-ethylenedioxy-20(S)-camptothecin,
7-Benzylaminomethylene-10, 11-methylenedioxy-20(S)-camptothecin, or
7-(3,4-Dimethoxybenzyl)-aminomethylene-10, 11-methylenedioxy-20(S)-camptothecin.

22. A process for preparing a compound of formula (I) as defined in Claim 1 or a pharmaceutically acceptable salt or solvate thereof which comprises:

(A) reacting a compound of formula (IV)



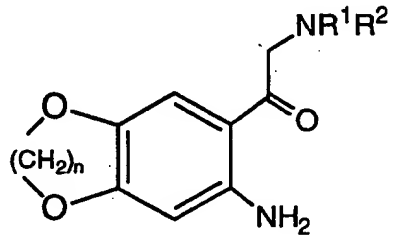
(IV)

wherein X is a leaving group, with a compound of formula (V)

$$HNR^1R^2 \quad (V)$$

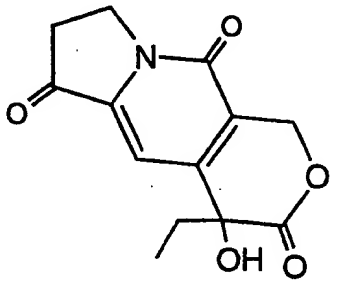
wherein R^1 and R^2 are as defined for formula (I); or

(B) reacting a compound of the formula (IIA)



(IIA)

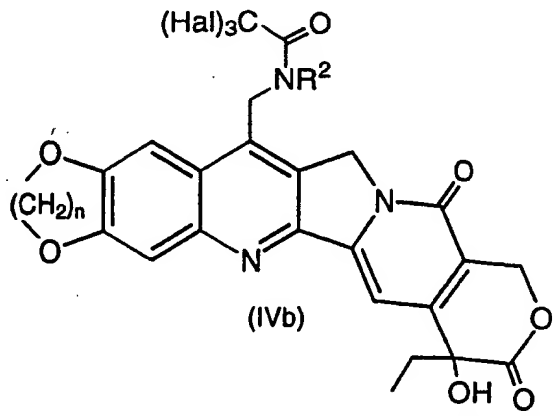
wherein R^1 and R^2 are as defined for formula (I), with a compound of formula (III)



(III)

or

(C) for the preparation of compounds of formula (I) wherein R^1 is hydrogen, reacting a compound of formula (IVb)



(IVb)

wherein Hal is halogen and R^2 is as defined for formula (I), with an acid to yield a salt of a compound of formula (I); and if necessary and/or desired subjecting the compound thus obtained to one or more further reactions comprising:

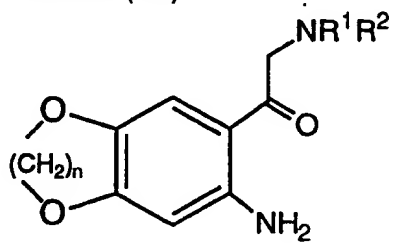
i) converting the resulting compound of formula (I) or a salt or a solvate or a protected derivative thereof into another compound of formula (I) and/or

ii) removing any protecting group or groups and/or

iii) converting a compound of formula (I) or a salt or solvate thereof into a physiologically acceptable salt or solvate thereof.

23. A compound of Claim 1 wherein the E ring is open.

24. A compound of the formula (IIA):



(IIA)

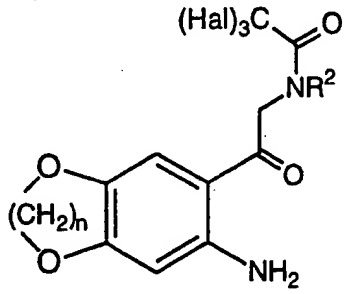
wherein n represents the integer 1 or 2; and

(i) R^1 represents hydrogen, lower alkyl, (C_{3-7})cycloalkyl, (C_{3-7})cycloalkyl lower alkyl, lower alkenyl, hydroxy lower alkyl, amino lower alkyl, lower alkylamino lower alkyl, amino lower alkyl, lower alkoxy lower alkyl or $(CH_2)_tAr$ wherein: t is 0 to 5 and Ar represents phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl; or phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl with one or more substituents independently selected from hydroxy, methoxy, halogen and amino; and

R^2 represents diphenylmethyl or $(CH_2)_tAr$; or

(ii) R^1 and R^2 taken together with the linking nitrogen represent N-tetrahydroquinolyl or N-tetrahydroisoquinolyl;
or a salt or solvate thereof.

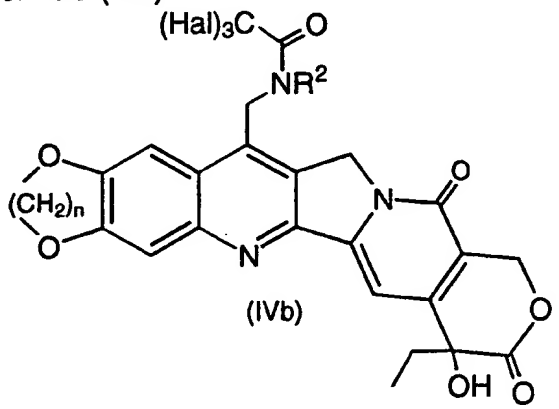
25. A compound of formula (IIb)



(IIb)

wherein n represents the integer 1 or 2, Hal is halogen and R^1 represents hydrogen, lower alkyl, (C_{3-7})cycloalkyl, (C_{3-7})cycloalkyl lower alkyl, lower alkenyl, hydroxy lower alkyl, amino lower alkyl, lower alkylamino lower alkyl, amino lower alkyl, lower alkoxy lower alkyl or $(CH_2)_tAr$ wherein: t is 0 to 5 and Ar represents phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl; or phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl, with one or more substituents independently selected from hydroxy, methoxy, halogen and amino, or a salt or solvate thereof.

26. A compound of formula (IVb)



(IVb)

wherein n represents the integer 1 or 2, Hal is halogen and R^2 is diphenylmethyl or $(CH_2)_tAr$ wherein: t is 0 to 5 and Ar represents phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl; or phenyl, furyl, pyridyl, N-methylpyrrolyl, imidazolyl, with one or more substituents indepently selected from hydroxy,

methoxy, halog n and amino, or a salt or solvate thereof.

INTERNATIONAL SEARCH REPORT

Inter mal Application No
PC I/US 94/04681

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07D491/22 C07D317/66 C07D319/18 A61K31/47 /
/(C07D491/22,317:00,311:00,221:00,221:00,209:00),(C07D491/22,
319:00,311:00,221:00,221:00,209:00),(C07D491/22,317:00,221:00,
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF MEDICINAL CHEMISTRY. vol. 34, no. 1 , 1991 , WASHINGTON US pages 98 - 107 W. D. KINGSBURY ET AL 'Synthesis of water-soluble (aminoalkyl)camptothecin analogues: Inhibition of topoisomerase I and antitumor activity' see page 98; table II ---	1,17,20
A	EP,A,0 418 099 (RESEARCH TRIANGLE INSTITUTE) 20 March 1991 cited in the application see claims 1,18,37 ---	1,17,20
P,X	EP,A,0 540 099 (GLAXO) 5 May 1993 see claims 1,9,11,18 --- -/--	1,17,20, 24

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 1994	27.07.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Voyiazoglou, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 94/04681

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 221:00,209:00),(C07D491/22,319:00,221:00,221:00,209:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EP,A,0 556 585 (TEKEDA CHEMICAL INDUSTRIES) 25 August 1993 see claims 1,28,33	1,17,20

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 1994	

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Voyiazoglou, D

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

.ernational application No.
PCT/US 94/ 04681

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK : ALTHOUGH CLAIMS 18, 19 ARE DIRECTED TO A METHOD OF TREATMENT OF (DIAGNOSTIC METHOD PRACTISED ON) THE HUMAN/ANIMAL BODY SEARCH HAS BEEN CARRIED OUT AND BASED ON THE ALLEGED EFFECTS OF THE COMPOUND/COMPOSITION.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ɔnal Application No
PCT/US 94/04681

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0418099	20-03-91	US-A-	5049668	17-09-91
		US-A-	5180722	19-01-93
		CA-A-	2066780	16-03-91
		JP-T-	5502017	15-04-93
		AU-B-	640950	09-09-93
EP-A-0540099	05-05-93	AP-A-	306	28-01-94
		AU-A-	2738592	06-05-93
		CA-A-	2081580	30-04-93
		JP-A-	5222048	31-08-93
		CN-A-	1074908	04-08-93
EP-A-0556585	25-08-93	CA-A-	2087898	25-07-93